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Evaluation of equine faecal inoculum with colonic inoculum for use in *in vitro* gas production.

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Introduction

In vitro gas production techniques to investigate the rumen or hindgut require samples representative of non-surgically modified healthy animals. Cannulation in horses has been shown to increase digesta passage rate (Austbø *et. al*., 2003) especially if the location of the fistula reduces the ability of the ventral colon to retain larger particles (Drogoul *et. al.,* 2000). Furthermore, cannulation is becoming no longer acceptable on ethical grounds, requires surgical facilities and a high level of management. There are few investigations that have assessed faecal inoculum as representative of rumen or large intestinal inoculum using the *in vitro* gas production technique.

Mauricio *et al.,* (2001) reported a high correlation between rumen liquor and faeces from two fistulated cows for OMD. However, they found lag times longer for faecel inoculum than for rumen inoculum. Harris *et al.,* (1995) reported that for all foods tested faecal material was a suitable alternative to rumen liquor, as long as separate calibrations were made for *in vivo* results. Lowman *et al.,* (1996) compared equine faeces with material from caecal fistulas of the same animals as inoculum for *in vitro* gas cumulation. Correlation values of r^2 values > 0.96 for gas production were obtained, VFA production was also similar as was DMD for all feeds tested when incubated with caecal or faecal inoculum. However, very limited literature is available on the comparison of equine faecal with colonic inoculum for use in *in vitro* gas production.

The colon is the main site of digestive disorders (Drogoul *et. al*., 2000) with soluble carbohydrates and undigested starch having a greater impact on the microbial communities in the colon than the caecum (de Fombelle *et. al*., 2003). Drogoul *et. al*., (2000) reported that the colon represented at least 80% of total gut MRT, therefore it can be assumed that digesta residing there would have a much greater effect on the microbial communities than in the caecum where MRT is shorter. Thus the colon is an important site for horse health. This work aims to compare faecal inoculum with colonic inoculum using *in vitro* gas production. If comparable, faeces could be used as a source of inoculum negating the need for surgically modified animals.

Method

Samples from the ventral colon and rectum were collected from four slaughter horses immediately after exsanguination. Immediately upon arrival, samples were vigorously mixed with buffer and strained through muslin before being added to bottles containing buffer and substrate (oats and medium quality meadow hay at 1 g / bottle on a 30:70 DM ratio). All procedures were carried out under anaerobic conditions at 38°C. Incubation was for 72 h with gas readings taken at 2, 4, 8, 12, 16, 20, 24, 30, 36, 48, 72 h using the semi-automatic gas production technique.

Results

No significant difference was found between sources of inocula or source time interaction. Cumulative gas production appeared most similar at 30-36 h, with greatest differences apparently at 16 and 72 h (Figure 1).

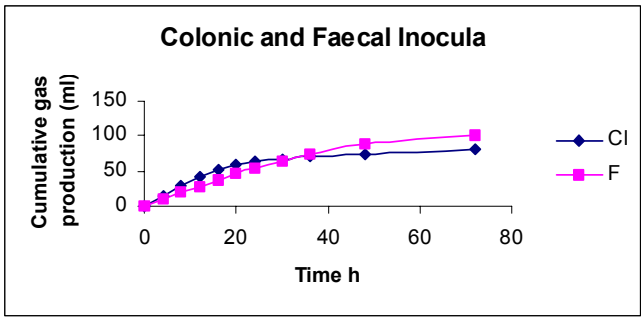

Figure 1: Cumulative gas production (ml)

Faecal inoculum had a slower rate of gas production than colonic inoculum up to 16h. After this time, colonic inoculum produced the higher rate of gas production (Figure 2). Faecal inoculum had a trend towards a longer lag phase than colonic inoculum by gas produced at 4 h (9.61 ml and 15.36 ml respectively). Total gas produced was lower for colonic inoculum than faecal inoculum (81.14 ml and 101.14 ml respectively).

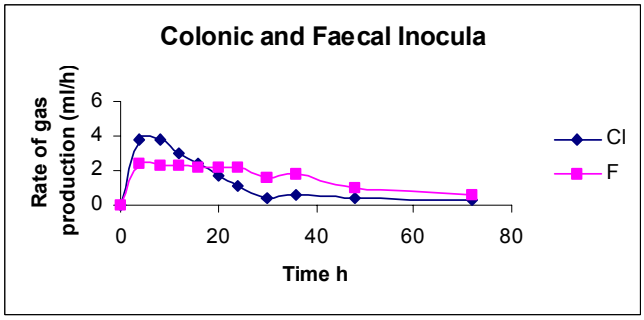

Figure 2: Rate of gas production (ml)

Discussion

Results indicate that faecal inoculum is a valid alternative to colonic inoculum. However, differences were noted between the two inocula which would require correction when using faeces. de Fombelle *et. al.* (2003) reported a significant difference between total number of anaerobes in the right ventral colon (rvc) and faeces which may account for some differences. However, the rate of gas production was not consistently lower for one particular inoculum, therefore total numbers of microbes is unlikely to be the only difference.

A higher rate of gas was produced by colonic inoculum during 0-16 h when predominantly starch and soluble fractions are fermented by e.g. lactobacilli and streptococci. This implies higher numbers of these microbes are present in colonic than faecal inoculum. de Fombelle *et. el.,* (2003) reported a direct relationship between communities in the rvc and starch in the diet. Therefore, it would be expected to find quantities of these starch-utilisers. Faecal communities in the horse however are unlikely to be exposed to starch. Therefore starch-utilisers would not be expected in numbers such as in the colon, as confirmed by the lower rate of gas produced from the faecal inoculum.

Less gas is produced at 16 h onwards by colonic inoculum than faecal inoculum. This suggests lower numbers of cellulolytics in colonic inoculum than faecal inoculum in contrast to that reported by de Fombelle et. al. (2003) in whose study no significant difference was observed between the two sites. As less cellulose would be expected

to be found in the faeces than the colon, this is unexpected data and requires further work. However, previous diets of the horses could be a contributing factor.

A trend towards a longer lag phase from faecal inoculum was measured by production at 4 h. Mauricio *et. al.,* (2001) described faecal microbial communities as having a 'survival mode' which may account for the longer lag phase. However, total gas production was highest from faecal inoculum. This is not surprising as faecal inoculum produced a more consistent rate of production than colonic inoculum.

Future work

Although there was no significant difference between the inocula, further work is required to understand and calibrate for the differences. Analysis of digestibility and volatile fatty acid profiles will give further information on function and molecular analysis will give information on community profiles.

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